a few lumps in it, and had a label in faded ink that read, "Dirt containing anthrax from La-Crosse outbreak. Aug. 1902, Dirt dried at 38 C," with the initials EGH (for E. G. Hastings) in one corner. We suspended 0.5 g of the "dirt" in 20 ml of sterile saline, and then injected 1 ml of the suspension intraperitoneally into each of five guinea pigs. One of the guinea pigs died in about 48 hr, but the others survived. Smears made from the heart blood, liver, and spleen of the dead guinea pig revealed large, capsulated, gram-positive, rod-shaped organisms. Cultures on Trypticase Soy Agar (BBL) slopes were made from the heart blood, and incubated for 18 hr at 37 C. The growth was typical of *Bacillus anthracis*. A suspension of these cells was made by washing one of the slopes with 5 ml of saline and injecting 1 ml intraperitoneally into each of three guinea pigs. These animals were dead within 48 hr, and smears from the heart blood, liver, and spleen contained large, capsulated, gram-positive, rod-shaped organisms. Further study of the culture, by the methods described by Leise et al. (J. Bacteriol. **77**:655, 1959), showed that it was nonhemolytic on sheep blood agar, and gave a positive "string-of-pearls" reaction. The cells were nonmotile. Subsequently, we sent a transplant of the culture to K. L. Burdon (Baylor University College of Medicine, Houston, Texas), who subjected it to a number of differential tests (Burdon, J. Bacteriol. **71**:25, 1956) and concluded that it was "a typical, virulent culture of *B. anthracis*."

Although the wording on the label of the bottle of soil might be interpreted to mean that a culture of *B. anthracis* isolated during an outbreak of anthrax at LaCrosse in 1902 had been added to dried soil at some subsequent time, this is not true. Both W. C. Frazier and E. B. Fred of this Department knew that Professor Hastings had a bottle of dried soil from a region where an anthrax outbreak had occurred, and that he could isolate *B. anthracis* from this material. Moreover, Professor Frazier stated that in 1920 he used soil from this same bottle to inject into guinea pigs to demonstrate anthrax for a class.

From the information given, it is concluded that *B. anthracis* survived for 60 years in dried soil kept at room temperature that varied from 50 to 100 F.

PLAQUE MORPHOLOGY OF MONKEYPOX VIRUS AS AN AID TO STRAIN IDENTIFICATION

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Received for publication 25 September 1963

The ability of the poxviruses to form plaques on monolayers of susceptible tissue cells, and the differentiation of variola from other members of the group by plaque morphology, has been reported by other investigators (Noyes, Proc. Soc. Exptl. Biol. Med. **83**:426, 1953; Younger, J. Immunol. **76**:288, 1956; Porterfield and Allison, Virology **10**:233, 1960; Mika and Pirsch, J. Bacteriol. **80**:861, 1960). In the course of identifying a newly isolated strain of monkeypox virus (McConnell et. al., Nature **195**:1128, 1962), it was noted that the plaque size of this isolate was smaller than that of a commercially available strain (Com Vac) of vaccinia vaccine virus (smallpox vaccine lot no. 5124A14, The National Drug Co., Philadelphia, Pa.). This finding suggested an additional possible method for differentiation of vaccinia strains of poxvirus by their plaque characteristics.

The Dulbecco plaque technique (Dulbecco, Proc. Natl. Acad. Sci. U.S. **38**:747, 1952), modified in minor detail, was employed. Monkey kidney cell cultures (MKCC) and rabbit kidney cell cultures (RKCC) were grown in 4-oz prescription bottles, essentially as described by Melnick and Riordan (Proc. Soc. Exptl. Biol. Med. **81**:208, 1952). Several strains (obtained

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TABLE 1. *Differential plaque morphology of representative poxviruses in both monkey kidney and rabbit kidney cell cultures*

Poxvirus Strain	Range of plaque sizes (in mm) 8 days postinoculation							
	Rabbit kidney cell culture				Monkey kidney cell culture			
Vaccinia (IHD)	3–6	4–6	4–6	ND[a]	5–7	4–7	4–7	4–8
Vaccina (D-Vac)	5–8	5–8	4–7	ND	4–7	4–6	3–5	3–6
Vaccinia (Com Vac)	4–6	3–6	3–5	ND	5–7	4–7	4–7	4–7
Vaccinia (Minnesota)	5–7	3–7	3–7	ND	2–4	2–3	2–4	2–4[b]
					5–6	4–6	5–8	5–7
Cowpox (red pack variant)	4–6	4–6	3–4	ND	4–7	5–7	0[c]	0
Cowpox (white pack variant)	2–5	3–4	2–3	ND	2–3[d]	4–6	0	0
Rabbitpox (UTRECHT)	5–7	5–7	4–7	4–7	3–6	3–5	3–6	3–5
Monkeypox (7-61 strain)	2–3	2–3	2–3	2–3	2–3	1–3	1–3	2–3

[a] Not done.
[b] Two distinct plaque sizes present.
[c] No readings, due to loss of virus.
[d] Last read on day 6. No further reading could be made.

through the courtesy of Leonard A. Mika, Fort Detrick, Frederick, Md.) of vaccinia virus (IHD, D-Vac, Minnesota), cowpox virus (red and white pock variants), and rabbitpox virus (UTRECHT), as well as commercial vaccinia and monkeypox (7-61 strain, Walter Reed Army Institute of Research) virus were plaqued simultaneously on both MKCC and RKCC. Seed virus for the plaque study was first-passage rabbit kidney cell culture material, stored in 1-ml quantities at −70 C until needed. All plaque trials were made from the same pool of seed virus.

Repeat plaquings of each strain of poxvirus were made, and the bottles were examined daily for time of appearance and size and shape of plaques. Only those bottles containing 3 to 30 plaque-forming units were measured (Table 1).

The size range for each strain of poxvirus stayed within close limits when repeated on the same cell system; however, some differences were noted when the second cell system was used. The Minnesota strain appeared to be a mixed infection, with two distinct plaque sizes (one 2 to 4 mm in diameter and the other 5 to 8 mm), when plaqued on MKCC. On rabbit kidney, the two sizes blended into one range, 3 to 7 mm in diameter. The size of the monkeypox virus plaques was relatively constant in both tissue culture systems, the plaque size being considerably smaller than those formed by the other species, except for the smaller variant of the Minnesota strain and the white cowpox variant. There was some suggestion that the maximal plaque size of IHD and Com Vac is smaller in RKCC than in MKCC, but this was not as obvious and pronounced as the reduction in maximal plaque size of rabbitpox in MKCC. The plaque size of the monkeypox strain was the most constant in the two cell systems. Plaques first appeared on the second day postinoculation for all strains except monkeypox virus, which appeared 1 to 2 days later. The time of appearance was the same for both cell systems. No other differences were observed in the plaque morphology of each strain of poxvirus.

The plaque technique is an exceptionally rapid and economical procedure available for routine differential diagnosis of poxvirus strains. The use of monkey kidney and rabbit kidney cell culture systems may be especially useful to investigators who do not utilize a CO_2 atmosphere for routine plaque work. This procedure may be especially helpful to those investigators handling large colonies of monkeys, a host susceptible to several strains of poxviruses.